Effects of moderate-intensity light on vitamin A-deficient rat retinas

Louvenia Carter-Dawson, Toichiro Kuwabara, and John G. Bieri*

The effects of moderate-intensity light (150 to 200 ft-cd) on retinal structure were compared between retinol-adequate and retinol-deficient rats after 1 to 6 days of light exposure during the 12 hr light phase of the cycle. Both damage to the outer segments and loss of photoreceptor cells were accelerated in retinol-adequate rats. Outer segments in retinas of retinol-adequate rats showed an abnormal staining pattern and disruption of disc structure in the distal portion about 2 days before those of retinol-deficient rats. After 4 days of exposure 24% of the photoreceptor cells had degenerated in the retinol-adequate retinas, but only 6% in the retinol-deficient retinas. By 6 days 65% and 41% of the photoreceptors had degenerated in the retinol-adequate and retinol-deficient retinas, respectively. Thus light exposure induced more rapid degeneration of photoreceptor cells in rats receiving adequate retinol than in those deficient in this vitamin.

Key words: light damage, vitamin A deficiency, photoreceptor degeneration, outer segments, retina, rat

Albino rats exposed to light of moderately high intensity develop abnormal electroretinograms (ERGs) followed by degeneration of photoreceptor cells.1–4 Similar changes can be induced in the rat retina by retinol deficiency.5–8 A combination of light damage and retinol deficiency might be expected to induce an abnormal ERG sooner than either condition alone. Physiological studies have shown that the ERGs of retinol-deficient rats are less affected by light than those of retinol-adequate rats.7, 9 Retinol deficiency appears to provide some protection against the loss of retinal function induced by exposure to light. In view of these findings, we have designed experiments to determine whether retinol deficiency provides protection against the morphological changes induced by light and whether the deficiency also alters the rate of photoreceptor degeneration after exposure to light.

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Materials and methods

Animals. Twenty male Sprague-Dawley rats were weaned at 21 days of age and divided into two groups: group I receiving basal diet supplemented with retinoic acid (4 mg/kg of diet) at 35 days of age and group II receiving basal diet supplemented with retinyl palmitate (4 mg/kg of diet) at weaning. The supplement was delayed in group I to prevent the sparing of tissue retinol by retinoic acid. The rats were caged individually and maintained on their respective diets at 25°C with 12 hr cyclic illumination of 10 ft-cd for 6 to 7 weeks.

Plasma retinol levels were determined at weekly intervals with a high-performance liquid chromatography procedure. At 6 to 7 weeks, when serum levels of retinol were quite low in group I (<6 μg/dl vs. control levels of 15 μg/dl), four rats from groups I and II were exposed to 150 to 200 ft-cd incident illumination for 1 to 7 days during the light phase of the 12 hr dark/12 hr light cycle.

Light was supplied by three cool-white Circline fluorescent bulbs surrounding a circular cage. A white plastic diffuser was inserted between the cage and the lights to provide relatively even illumination inside the cages. The level of illumination was measured in different positions inside the cage with the diffuser; thus 150 to 200 ft-cd represent the range.

Five minutes before the end of the 12 hr exposure the cage lights were turned off. The rats spent the remaining 3 to 4 min in 10 ft-cd of light. They were killed after 12 hr of darkness and 3 to 3.5 hr into the light phase (rats in 10 ft-cd of light) of the 12 hr light/12 hr dark cycle. Fans were used to keep the cage temperature between 26° to 30° C.

Histological procedures. For histological analysis, eyes were enucleated and fixed by immersion in 4% glutaraldehyde in 0.15M Na-K phosphate buffer for 45 to 60 min. Portions of the posterior inferior nasal retina were excised within 2 mm of the optic nerve head. The segments were post-fixed with 1% osmium in Na-K phosphate buffer for 30 min at room temperature and 30 to 45 min at 4°C. The tissue was dehydrated in a graded series of alcohol and processed for embedding in Epon.

Sections for light microscopy were cut at 1 to 1.5 μm and stained with a solution of 1% toluidine blue in 1% tetraborate buffer. Ultrathin sections stained with uranyl acetate and lead citrate were examined with an electron microscope.

Counts of photoreceptor nuclei were made within 2 mm of the optic nerve head in the posterior retina of the inferior hemisphere. In this region of retina, four consecutive segments 128 μm in length were examined in each of four sections (two to four animals). The number of photoreceptor nuclei was recorded, and the mean, S.E.M. among animals, and percentage of remaining nuclei calculated.

Results

After 1 day of cyclic light exposure (12 hr of exposure), microscopic changes were evident in the retinas of retinol-adequate rats. With toluidine blue, the distal portion of the outer segments stained abnormally lighter than the proximal portions (Fig. 1, C). In contrast,
Fig. 1. For legend, see opposite page.
Fig. 2. Electron micrographs of photoreceptor outer segments from retinol-adequate and retinol-deficient rat retinas after 4 days of light exposure. (×9600.) A, Retinol-adequate. Outer segments are swollen, and the discs are disrupted into numerous vesicles (arrow). B, Retinol-deficient. Some outer segment discs are disrupted into vesicles but are interspersed with groups of intact discs (arrows). The pigment epithelial processes have lost the intimate contact with photoreceptor outer segments as a result of the deficiency. pep, Pigment epithelial processes.

Ultrastructural differences were also seen between photoreceptor outer segments in retinol-adequate and retinol-deficient rats after exposure to light. Some discs in the photoreceptor outer segments of retinol-adequate rats were distended, and others appeared ruptured into vesicles as early as 2 days of light exposure. After 4 days, discs in the outer segment tips from retinol-adequate rats were severely affected. Some discs were apparently ruptured into large vesicles with a maximum diameter of 0.4 μm. The outer segments were swollen and showed an increase in diameter of about 0.3 μm (retinol-adequate 1.4 ± 0.05 μm; retinol-deficient 1.1 ± 0.02) (Fig. 2, A). In the retinol-deficient rats some discs were apparently dispersed into small vesicles 0.1 to 0.2 μm in diameter after 4 days of exposure but were interspersed with groups of intact discs (Fig.
Table I. Number of photoreceptor cells* in sections of retinas from retinol-adequate and retinol-deficient rats in different lighting conditions

<table>
<thead>
<tr>
<th></th>
<th>Unexposed</th>
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<td></td>
<td>0 days</td>
<td>2 days</td>
<td>4 days</td>
<td>6 days</td>
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<tr>
<td>Retinol-adequate:</td>
<td></td>
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<tr>
<td>Rods and cones</td>
<td>148.0 ± 7.0</td>
<td>132.5 ± 2.6</td>
<td>112.3 ± 7.0</td>
<td>51.0 ± 5.5</td>
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<td>% Rods and cones remaining</td>
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<td>89.5</td>
<td>75.7</td>
<td>34.5</td>
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<tr>
<td>Retinol-deficient:</td>
<td></td>
<td></td>
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<tr>
<td>Rods and cones</td>
<td>137.0 ± 2.0</td>
<td>122.0 ± 4.2</td>
<td>128.7 ± 6.9</td>
<td>80.2 ± 1.9</td>
</tr>
<tr>
<td>% Rods and cones remaining</td>
<td>100</td>
<td>89.1</td>
<td>93.9</td>
<td>58.5</td>
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*Mean ± S.E.M. for 2 to 4 rats in 128 μm of posterior retina.

2, B). Discs in photoreceptor outer segments of retinol-deficient rats were affected to a lesser extent at 4 days of exposure.

In addition to the difference in outer segment structure in retinol-adequate and retinol-deficient rats after exposure to light, the rate of photoreceptor cell degeneration also differed (Table I). At 2 days of light exposure a small number of photoreceptors were lost in both groups. By 4 days, 76% of the photoreceptors remained in the retinol-adequate rats, but about 94% of the photoreceptor cells remained in the retinol-deficient group. After 6 days, only about 35% of the photoreceptors remained in the retinol-adequate retinas, but about 59% remained in the retinol-deficient group. Photoreceptor cells thus remained longer in the retinas of rats deficient in retinol.

Discussion

Light of moderate intensity causes retinal damage which varies according to dietary retinol. The underlying mechanism or mechanisms which lead to retinal damage are not clearly understood. Noell and Albrecht showed that the ERGs of rats deficient in retinol were affected to a different extent by light. Before exposure, the A and B waves of the ERGs in the deficient rats were reduced in amplitude. At the end of a 40 hr exposure, rats in both groups were given a high dose of retinol, and the ERGs were tested 10 days later. The A and B waves of the retinol-adequate rats were as high as those of retinol-adequate rats before exposure, indicating that the retinol-deficient retinas had escaped severe injury.

Our morphological data provided evidence that light injures photoreceptor outer segments less in retinol-deficient retinas. Outer segments of retinol-adequate rats lost the normal staining pattern as early as 2 days of light exposure. This pattern of staining was seen initially at the outer segment tips but, with increasing exposure, extended to more proximal regions. The outer segments in the retinol-deficient retinas showed a loss of normal staining but only after 6 days of light exposure.

The abnormal staining pattern seen in the outer segments after exposure to light appears related to disc structure. Portions of the outer segments showing the abnormal staining pattern contained discs that were distended and apparently broken into vesicles of various sizes. Initially, discs were damaged in the distal portion of the outer segment, but with longer exposures disc structure was abnormal in the proximal region. The pattern of disc disruption paralleled the abnormal staining pattern.

Photoreceptor nuclei also degenerated at a different rate in retinol-deficient and retinol-adequate rats after exposure to light. By 4 days of light exposure, about 76% of the photoreceptor nuclei remained in retinol-adequate rats, but approximately 94% remained in the retinol-deficient rats. Similarly, at 6 days, 35% and 59% persisted in retinol-adequate and retinol-deficient rats, respectively. Photoreceptor cells degenerated at a
faster rate in the retinol-adequate rats after exposure to light.

The precise basis for the "protection" from retinal damage induced by light in retinol-deficient rats is not clear. However, Noell and Albrecht7 and Noell9 have shown that the ERGs in retinas that contain less rhodopsin as a result of dim illumination or vitamin A deficiency are affected to a lesser extent by light exposure than those containing more rhodopsin. It has also been reported that a larger percentage of photoreceptor cells degenerate in retinas which contain higher levels of rhodopsin when exposed to light of high intensity.12 There is a strong correlation between the amount of retinal damage induced by light and the level of rhodopsin. Thus lower levels of rhodopsin may provide a basis for protection against light damage.

Altered levels of hormones induced by vitamin A deficiency may be involved in protection against light damage. Recent studies have shown that removal of the pituitary or ovaries reduces significantly the amount of retinal damage induced by light.13,14 Olafson and O'Steen13 and O'Steen14 concluded that hormones secreted by the pituitary, as well as pituitary target organs, exert a regulatory influence on the severity of light-induced retinal damage. In retinol-deficient male rats testosterone levels are slightly reduced but not significantly lower than controls, and the pituitary gland showed an increase in size and number of gonadotrophic cells,15 as well as increased gonadotrophic activity.16 On the basis of these results it would appear that hormones are produced by the pituitary as well as the testes in retinol-deficient male rats. The possibility that an altered level of individual hormones is involved in the "protection" against light damage in the retinol-deficient rat cannot be eliminated without further study.

REFERENCES